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Tennessee Valley Authority, Muscle Shoals, AL

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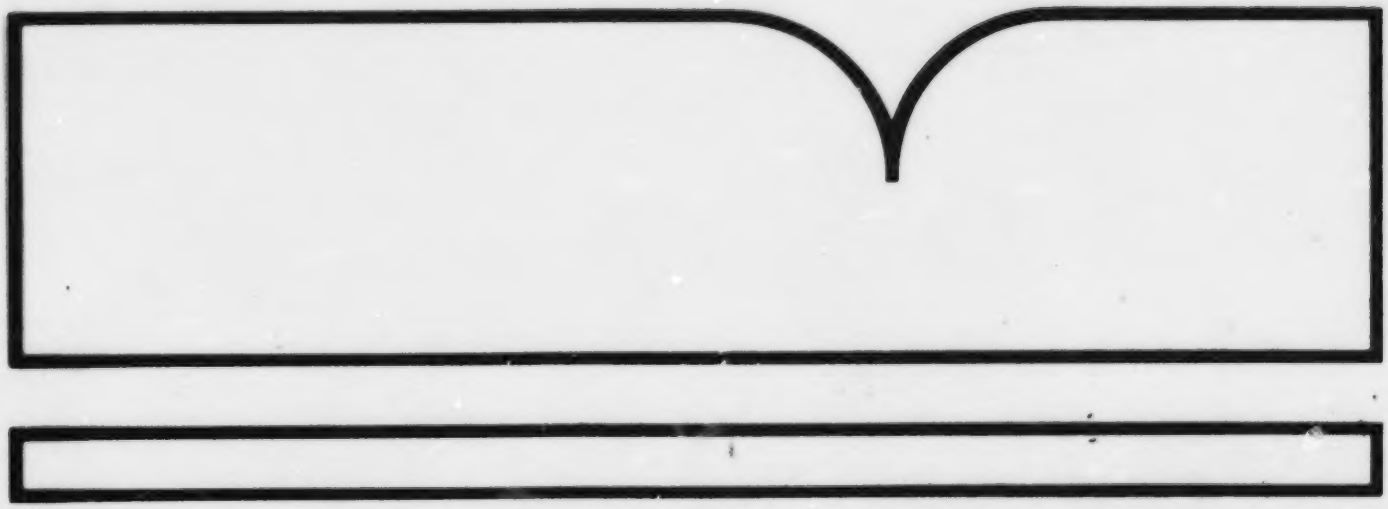

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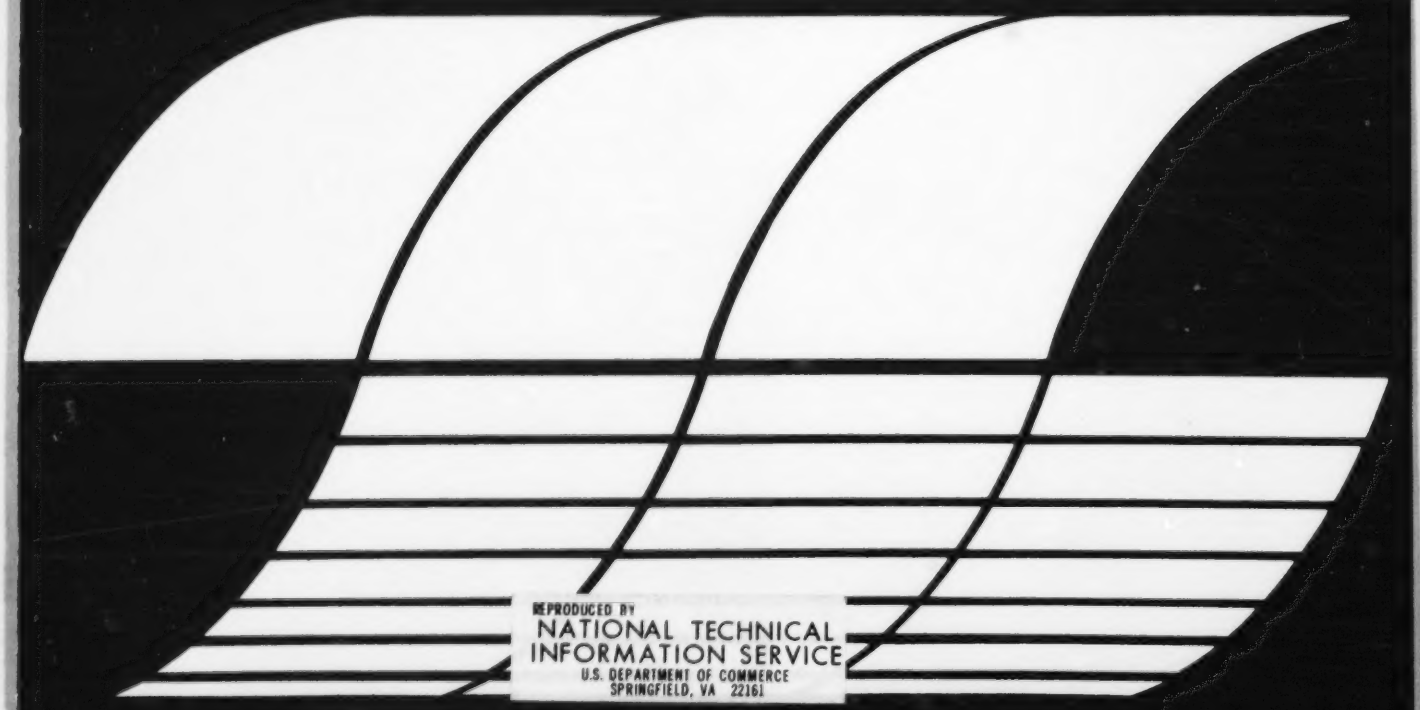

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THERMAL EFFECTS ON BLUEGILL HEMATOLOGY

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ABSTRACT

Temperature effects on hematological responses of adult bluegill were examined for constant vs fluctuating temperature regimes, for ambient vs high temperature, and for intake vs discharge temperatures at an operating power plant. Multivariate statistical methods were used to determine temperature effects. Different hematological responses were observed between different constant temperature regimes, between different fluctuating temperature regimes, and between ambient and high temperatures, but not between intake and discharge areas of a power plant. Red blood cell roundness increased by 11-15 percent with the higher temperatures, but only accounted for approximately 17 percent of the total variation on the average. The hematological responses were complex in all cases with 5 to 10 variables contributing to at least 80 percent of the total variation.

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SECTION 1

INTRODUCTION

Use of fish blood is currently recognized as a potential index of fish response to water quality because it is sensitive to a variety of environmental conditions (Block 1974, MacLeay 1975, Wedemeyer 1976). In a recent review Houston (1973) indicated an increasing appreciation of the potential effect of temperature on ionic and osmotic regulation in teleosts. In addition to observed changes in plasma osmolality, changes in hematocrit, hemoglobin, and certain electrolytes have been observed in some fish subjected to temperature stresses (Cameron 1970, DeWilde and Houston 1967, Falkner and Houston 1966, Heinicke and Houston 1965, Houston and DeWilde 1968, 1969, Houston et. al 1970, Houston and Madden 1968, Parvatheswararao 1967, Reaves 1968). Changes in the blood cellular elements have also been observed following thermal stresses, particularly the abundance of erythrocytes, and the distribution of lymphocytes and eosinophils (DeWilde and Houston 1967, Houston and DeWilde 1969, Pickford et. al 1971). It is evident from the literature that there are differences in hematological responses between fish species. However, data on the thermal effects to any one fish species are relatively sparse.

Data are also meager on blood composition associated with temperature pulsing. There is some evidence, however, that constant and fluctuating (diurnal) temperatures do not always effect similar physiological responses (Toews and Hickman 1969). The type and intensity of thermal stress are major considerations in locating morphological, physiological, and biochemical changes in fish blood. A challenging aspect of the problem is to find a response mechanism of the blood that is sensitive to relatively low thermal intensity, such as thermal gradients associated with power plant discharge plumes.

The purpose of this study was to examine temperature effects on hematological responses in adult bluegills. In particular, hematological responses were examined in relation to: (1) constant and fluctuating temperature regimes, and (2) ambient vs high temperature and (3) intake and discharge temperature regimes at an operating power plant.

SECTION 2

CONCLUSIONS

Bluegill hematology was examined to determine effects of temperature. Hematological responses were different for fluctuating temperature regimes compared to those of constant temperature regimes. An increased erythrocyte fragility was associated with fluctuating temperature regimes as well as with the suboptimal temperature regime. Thrombocytosis with a concomitant leukopenia was a common observation for the higher fluctuating temperature as well as for the higher acclimating temperatures. Reduced erythrocytic sedimentation rates were observed for the higher temperatures. Although hematology may vary depending on fish size, good hematological indicators of potential thermal stress were associated with red blood cell morphology, particularly cell size and/or shape. However, in situ hematological responses of bluegill at an operating power plant were apparently not affected by the associated thermal gradients.

SECTION 3

RECOMMENDATIONS

Based on the complexity of hematological responses, it is recommended that multivariate statistical methods be used to determine thermal effects. In view of the nonspecificity of the hematological response, in spite of good indicator cell morphology variables, it is further recommended that as many hematological values as possible be analyzed in the multivariate response.

SECTION 4

EXPERIMENTAL PROCEDURES

Bluegill, Lepomis macrochirus Rafinesque, were obtained from Carbon Hill National Fish Hatchery, Carbn Hill, Alabama.

CONSTANT VS FLUCTUATING TEMPERATURE REGIMES

Fish were kept in five 400-gallon galvanized horse troughs which were pretreated with a temperature-resistant epoxy coating. Tanks were pre-seasoned and used for fish holding for approximately two years before this study. In this study about 30 fish were held in each tank for approximately five months. Standpipes were installed in each tank to allow for water flowthrough. Water turnover time was approximately 12 hours. Stainless steel coils were fitted to the tank interiors to allow for indirect temperature exchange. Cold water passed through one coil, hot through the other. Water flow through the coils was regulated by solenoid switches on clock timers to maintain desired water temperatures in the tanks. Submersible pumps, one per tank, circulated the water sufficiently to maintain constant temperatures in all areas of the tanks. Temperatures were monitored continuously with thermistors connected to a thermograph, and monitored every 15 minutes with calibrated thermometers suspended in each tank. Because of the difficulty in calibrating the thermograph to the desired accuracy, thermographs were used to ensure there was no temperature stratification, whereas manual readings were sufficiently accurate to characterize the thermal profiles. Thermal profiles are presented in Figure 1. Five thermal profiles were maintained. Constant temperature profiles were as follows: Group 1 at mean of 17.1°C with a standard error of ± 0.01, group 3 at 21.7 ± 0.04°C, and group 5 at 26.7 ± 0.03°C. Fluctuating temperature profiles were set up at one of the constant temperatures for a 19-hour period, receiving a gradual temperature pulse over a 5-hour period once a day reaching the next constant temperature. Group 2 temperatures were 17.0°C with a temperature pulse reaching 22.2°C, and group 4 temperatures were 21.7°C with a temperature pulse reaching 26.2°C.

Fish were loaded into the tanks in November 1978, acclimated to constant temperatures for six weeks, then to experimental temperature regimes for two weeks. A 10-hour photoperiod was started with a 30-minute low-intensity incandescent light source, followed by a 9-hour room intenstiy fluorescent light source (approximately 20-ft candles at tank level), followed by a 30-minute low intensity incandescent light source, followed by a 14-hour dark period. Dechlorinated water was used. Water quality was similar for all tanks. Filters were changed daily to assure good

water quality. Dissolved oxygen was at saturation levels. Tanks were cleaned daily to avoid food and fecal accumulation on the tank bottoms. Fish were fed daily ad libitum a diet of trout chow. Fish were bled about 23 hours after their last feeding.

Peripheral blood samples were collected two weeks after the temperature pulsing began. Blood collection and most analytical methods are described elsewhere (Burton and Murray 1979, Murray and Burton 1979). In addition, leucocyte-thrombocyte differential counts were determined from a field of 200 cells and smudge cells from a field of at least 3,000 cells from blood smear slides.

Blood from 22 to 24 fish was examined for each group. Most of the fish in each group were males. Multivariate and univariate analysis of variance methods were used to determine effects of constant vs fluctuating temperatures.

AMBIENT VS HIGH TEMPERATURE

Adult bluegill, Lepomis macrochirus Rafinesque, were seined from ponds at Carbon Hill National Fish Hatchery and kept in concrete holding tanks for approximately 4 months. Fish were stocked at a biomass density of 3.8 kg/m^3, resulting in 1.1 fish cm/ℓ. Pond water was pumped into both tanks. Water replacement time of the 1,514 liter tanks was approximately 5 hours. Temperatures were monitored daily. The mean water temperature of fish kept at ambient temperature was 12.3 ± 1.9°C during the last 3 weeks of holding. The mean water temperature of fish kept at the higher temperature was 20.1 ± 1.8°C and was maintained by 4 immersion coils. Dissolved oxygen ranged from 6 ppm in the ambient temperature tank to 12 ppm in the high temperature tank. Although the light was somewhat subdued in the tank house, the fish were exposed to a natural photoperiod. Fish were fed ad libitum a daily diet of trout chow. Fish were bled 23 hours after their last feeding. Blood from twenty males was analyzed for each temperature treatment.

INTAKE VS DISCHARGE TEMPERATURES

Male bluegill, Lepomis macrochirus Rafinesque, were transported in aerated holding tanks from Carbon Hill National Fish Hatchery to John Sevier Power Plant, which is located near Rogersville, Tennessee. Fish were transferred into a wire mesh cage inside the intake area of the power plant and were acclimated to ambient water conditions for at least 2 weeks. At the end of the acclimation period, fish were separated into two groups. The control group was kept in the intake forebay of the power plant. The second group was held in a cage in the heated discharge. The fish were exposed to these conditions for 7-8 days. Fish in the discharge were exposed to elevated water temperatures and intermittent chlorination. The mean water temperature of the discharge area ($\bar{x}$ ± s.d = 31.3 ± 1.8°C) was approximately 7.5°C higher than that of the intake area (23.8 ± 1.6°C). The chlorination schedule of the 4-unit plant consisted of 13 pulses of about 10-60 minutes pulse duration. Since only one unit was chlorinated

at any one time, the chlorine levels in the discharge channel during chlorination were approximately 0.015-0.22 mg/ℓ free chlorine and 0.15-0.34 mg/ℓ total residual chlorine (amperometric titration method).

Both groups of fish were held at population densities of less than 0.60 cm/ℓ, which represents a desirable density (Lewis 1963). The fish were not fed additionally to their natural plankton diet during the acclimation and observation periods. At the end of the experimental period, each fish was pithed and peripheral blood was collected and analyzed as previously described (Burton and Murray 1979, Murray and Burton 1979).

SECTION 5

RESULTS AND DISCUSSION

CONSTANT VS FLUCTUATING TEMPERATURE REGIMES

Temperatures for growth of adult bluegill have been reported from 16-30°C with an optimum from 24-27°C (Brungs and Jones 1977). The lower end of the temperature spectrum is a more likely reference in this study since the fish were bled during the winter, viz. 17.1°C or group 1 for the constant temperature comparisons and 17.0-22.2°C or group 2 for the fluctuating temperature comparisons. The fluctuating temperature regimes were such that the baseline temperature was similar to one of the constant temperature regimes and the pulsed temperature was similar to the next higher constant temperature regime (Figure 1). The fluctuating temperature regime represented by group 2 is thus bound by the constant temperature regimes represented by groups 1 and 3. Similarly, at the higher temperatures the fluctuating temperature regime represented by group 4 is bound by the constant temperature regimes represented by groups 3 and 5.

In order to determine hematological similarities between the upper and lower end of the temperature spectrum, results of multivariate analysis of variance tests were compared for groups 1, 2, and 3, and groups 3, 4, and 5 (Table 1). Since the hematological variables that were affected were different for each group of temperatures (Table 2), separate analyses will follow. Four hematological variables were significantly different for the comparison between groups 1, 2, and 3. The mean erythrocytic volume (MEV) increased by approximately 13-16 percent (Table 3) with the upper temperature bound of the lower temperature regimes (group 3) regardless of constant or fluctuating temperatures (groups 1 and 2). Similarly, the mean erythrocytic hemoglobin (MEH) increased by approximately 12-17 percent with the higher temperature with no distinction between constant and fluctuating temperatures (groups 1 and 2) (Table 3). The number of smudge cells decreased by approximately 47-57 percent with the higher temperatures. Apparently the erythrocytes are very fragile at the suboptimum temperature. The number of neutrophils also decreased with the higher temperature by about 40 percent between groups 1 and 3. There was, however, a distinction in response between groups 1 and 2 where the neutrophil distribution decreased by about 46 percent with the fluctuating temperature regime, apparently responding to the higher pulse temperature in that temperature profile (Table 3). Ten variables accounted for 80.09 percent of the total variation. Indicator variables appear to be thrombocyte-leucocyte distribution, red blood cell morphology, and hematological indices (Table 4). Results of the partial correlations were not unusual (Table 5).

With the exception of neutrophil distribution no other hematological differences were observed between groups 1 and 2 (Table 2). In addition, it is clear from Table 1 (groups 1, 2) that the hematological response is similar between the fluctuating temperature regime and the lower constant temperature bound in the lower temperature regimes (groups 1, 2, 3). Eight hematological variables accounted for 84.01 percent of the total variation (Table 6). Indicator variables were associated with distribution of neutrophils and lymphocytes, red blood cell size and shape, sedimentation rate and hemoglobin levels of red blood cells. The partial correlations indicated in Table 7 were not unusual.

The hematological response was different for the two constant temperature regimes of 17.1°C and 21.7°C (Table 1; groups 1, 3). Ten hematological variables responded differently between the two groups (Table 2). The hematological response associated with the higher temperature (group 3) included lower erythrocytic sedimentation rates (by 26 percent) smaller erythrocytes (by 4-10 percent), decreased numbers of smudge cells (by 47 percent), decreased distribution of neutrophils (by 40 percent) and eosinophils (by 69 percent), with a consistant increase in thrombocyte distribution (by 8 percent), and increased mean erythrocyte volume (by 17 percent) and mean erythrocytic hemoglobin (by 18 percent) (Table 3). Very little information is available in the literature on temperature effects of these variables. One study examined the effects of erythrocytic sedimentation rates (ESR) of fish blood. In a comparative study between emaciated bluegill, captured from the discharge area of a power plant where temperatures reached 36°-40°C, and healthy bluegill, captured from cooler water of the same lake, the ESR of the emaciated fish in the discharge area was lower than that of the healthy fish in the cooler area of the lake (Graham 1974). Variable results have been reported for the mean erythrocytic volume and mean erythrocytic hemoglobin (Falkner and Houston 1966, Houston and DeWilde 1968, Catlett and Millich 1976). No results have been reported for leucocyte-thrombocyte distribution or red blood cell morphology. Ten variables accounted for 82.63 percent of the total variation (Table 8). Indicator hematological variables appear to be red blood cell size, shape, and sedimentation rate, leucocyte-thrombocyte distribution, hematological indices, and smudge cell abundance. The partial correlations indicated in Table 9 were not unusual.

Results indicate that the hematological response that is associated with the fluctuating temperature regime (17.1-21.7°C) is not similar to that of the higher constant temperature bound of the lower temperature regimes, viz 21.7°C (Table 1; groups 2, 3). Five hematological variables were different between groups 2 and 3 (Table 2). The magnitude of the differences and the direction of change (increase or decrease) were similar to those between groups 1 and 3 (constant temperature regimes) (Table 3). Therefore, when the data are stratified in this way, the only differences attributable to the fluctuating temperature regimes are those in hematological indices, distribution of eosinophils, abundance of smudge cells, and red blood cell shape. Seven hematological variables accounted for 82.26 percent of the total variation (Table 10). In addition to the five variables mentioned above, other indicator variables are cell size and

lymphocyte distribution. Results of the partial correlations were not unusual (Table 11).

There were significant differences in hematological response between constant and fluctuating temperature regimes at the higher end of the temperature spectrum (Table 1; groups 3, 4, 5). Responses of four hematological variables were significantly different between the three groups (Table 2). With the higher temperatures cell length decreased by 4.2-4.5 percent, cell width decreased by 7.6-8.8 percent, and cell roundness increased by 11.9-13 percent with no distinction between groups 4 and 5, viz. the fluctuating temperature and the upper constant temperature bound (Table 3). Seven hematological variables accounted for 83.32 percent of the total variation (Table 12). Indicator variables appear to be red blood cell morphology, abundance and sedimentation rates, abundance of smudge cells, and hematocrit, with hematocrit and cell roundness each accounting for at least 20 percent of total variation. The partial correlations, presented in Table 13, were not unusual.

Results indicate a distinct hematological response associated with the fluctuating temperature regime of 21.7-26.2^{o}C compared to the constant lower temperature bound of 21.7^{o}C (Table 1, groups 3 and 4) but not when compared to the constant upper temperature bound of 26.2^{o}C (Table 1, groups 4 and 5). The hematological response associated with the fluctuating temperature regime included less eliptically shaped or more round shaped (by 12 percent) red blood cells and an increased number of smudge cells (by 50 percent) when compared to that of the lower temperature bound (group 3) (Table 3). Five hematological variables accounted for 86.97 percent of the total variation with cell roundness accounting for 40 percent (Table 14). The hematological response is better defined, i.e. by less variables, in this data group than for the previous data groups. In addition to the variables mentioned above, eosinophil distribution is also a characteristic indicator of fluctuating temperature (21.7-26.2^{o}). Results of the partial correlations indicated in Table 15 were not unusual.

The hematological response of the fluctuating temperature (21.7-26.2^{o}) was similar to the upper constant temperature bound (26.7^{o}C) (Table 1, groups 4 and 5). Tables 2 and 3 show decreases in erythrocytic sedimentation rate (by 29 percent) and smudge cell abundance by (57 percent) with the higher temperature. Six variables accounted for the total variation, indicating a fairly well defined response for hematology. In addition to the hematological variables already mentioned, other indicator variables appear to be abundance of red blood cells and hemoblasts, mean erythrocytic hemoglobin, and hematocrit. Results of the partial correlations were not unusual (Table 17).

The difference in hematological response between the two constant temperature regimes (21.7^{o}C and 26.7^{o}C) included lower sedimentation rates (by 30 percent), erythrocytosis (by 12 percent), and more round (by 13 percent) red blood cells (Table 1, groups 3 and 5; Tables 2 and 3) with the higher temperature. These responses have some similarities with those at the lower temperature regimes (17.1^{o}C or 21.7^{o}C). Both of these

comparisons examine the hematological differences associated with a 5°C difference. A general hematological response observed for a 5°C difference is a reduced erythrocytic sedimentation rate of about the same order of magnitude, viz. 26-30 percent. A change in cell morphology was also a common observation. For the difference between the lower temperatures, cell area was affected; whereas, for the difference between the higher temperatures, cell shape was affected. Seven hematological variables accounted for 80.46 percent of the total variation (Table 18). In addition to the hematological variables already mentioned, indicator variables include mean erythrocytic hemoglobin and hematocrit. Partial correlations were not unusual (Table 19).

Significant changes in hematological response were also observed when the 5°C difference was between the fluctuating temperature regimes (Table 1, groups 2 and 4). With the higher temperature there was an increase in thrombocyte abundance (by 10 percent), a decrease in lymphocytes (by 18 percent), a decrease in eosinophil abundance (by 88 percent), a decrease in mean erythrocytic hemoglobin concentration (by 2 percent), and rounder red blood cells (by 14 percent) (Tables 2 and 3). Respiration was apparently not impaired however, because the hemoglobin values were virtually similar. Thrombocytosis was also observed between groups 1 (17.1°C) and 3 (21.7°C) as was eosinopenia, whereas changes in cell shape were also observed between groups 3 (21.7°C) and 5 (26.7°C). Six hematological variables accounted for 83.34 percent of the total variation (Table 20). Results of partial correlation analysis were not unusual (Table 21).

Thrombocytosis with a concomitant leukopenia was a common observation with increased temperature for fluctuating temperatures (groups 2 and 4) and for higher acclimating temperatures (groups 3 and 5). Fragile erythrocytes were consistently observed for fluctuating temperatures as well as for suboptimal temperatures (Table 3). Changes in red blood cell morphology also accompanied differences in temperature regimes; the most frequent observation being rounder cells. Reduced erythrocytic sedimentation rates were observed with differences in constant temperature regimes.

AMBIENT VS HIGH TEMPERATURE

Significant differences were observed in hematological response between ambient and high temperatures during the autumn months (Table 22 increased temperature). In Tables 23 and 24 the higher temperature shows decreased levels for mean erythrocytic hemoglobin concentration (by 32 percent), with no change in overall hemoglobin, rounder red blood cells (by 11 percent) with a compensating change in cell length (by 7 percent), thrombocytopenia (by 3 percent) with a concomitant lymphocytosis (by 27 percent). The response is somewhat generalized since seven hematological variables account for at least 80 percent of the total variation (Table 25). In addition to the above-mentioned variables, other indicator variables include cell width and cell height. Results of the partial correlations, indicated in Table 26, were not unusual.

Changes in red blood cell morphology seem to be consistant with changes in temperature (cf. previous study). However, the conflicting results of the thrombocyte-leucocyte distribution between the previous study and this study are not easily dealt with. The hematological response is somewhat nonspecific since the thrombocyte-leucocyte variables attributed the same order of magnitude (less than 10 percent each) to the total variation for each of the comparisons (Tables 4, 6, 8, 10, 12, 14, 16, 18, and 20).

INTAKE VS DISCHARGE TEMPERATURES

The results indicate no overall effect of temperature on hematological response at the power plant (Table 22 intake vs discharge) in spite of the hematological differences rendered by significant univariate analysis between the intake and discharge areas (Table 23). The results of the univariate analysis can, therefore, be misleading for multivariate determinations. Nevertheless, these results may be of value in determining indicator variables associated with temperature differences. Cell roundness appeared to be the best indicator variable accounting for 19.42 percent of the total variation (Table 27). Six hematological variables accounted for 80.19 percent of the total variation, three of them relating to cell morphology. Results of the partial correlations were not unusual (Table 28).

Reduced levels for hematocrit and hemoglobin have been observed in emaciated bluegill in the discharge area of a power plant (Graham 1974). Results of the present study tend to support these observations, except that the fish in the present study were not emaciated, as determined by stomach analysis. The data in the present study suggest that respiration may be impaired in those fish in the discharge area by the rounder red blood cells with concomitant reductions in MEHc, hemoglobin and hematocrit. These results (changes in hematocrit and hemoglobin values), however, do not appear to be gnomonic of temperature change in bluegill since they were not observed in the previous studies above. In the present study erythrocytopenia was observed, whereas in one of the studies above (22^{o}C vs 27^{o}C constant temperature regimes) erythrocytosis was observed. These conflicting results may be associated with fish size (cf. Banks et. al 1971).

The only consistent responses in this study with the previous studies involve changes in red blood cell morphology. In this study red blood cells were approximately 15 percent rounder in the fish in the discharge area compared to those in the intake area. Variations in red blood cell size and/or shape have also consistently been observed for crowding and intensive culturing (Murray and Burton 1979, Murray 1980). This knowledge, coupled with the results of the above studies indicate that, with respect to overall hematology, changes in red blood cell morphology associated with environmental perturbations appear to be nonspecific to the type of disturbance but appear to be excellent general indicators of environmental change.

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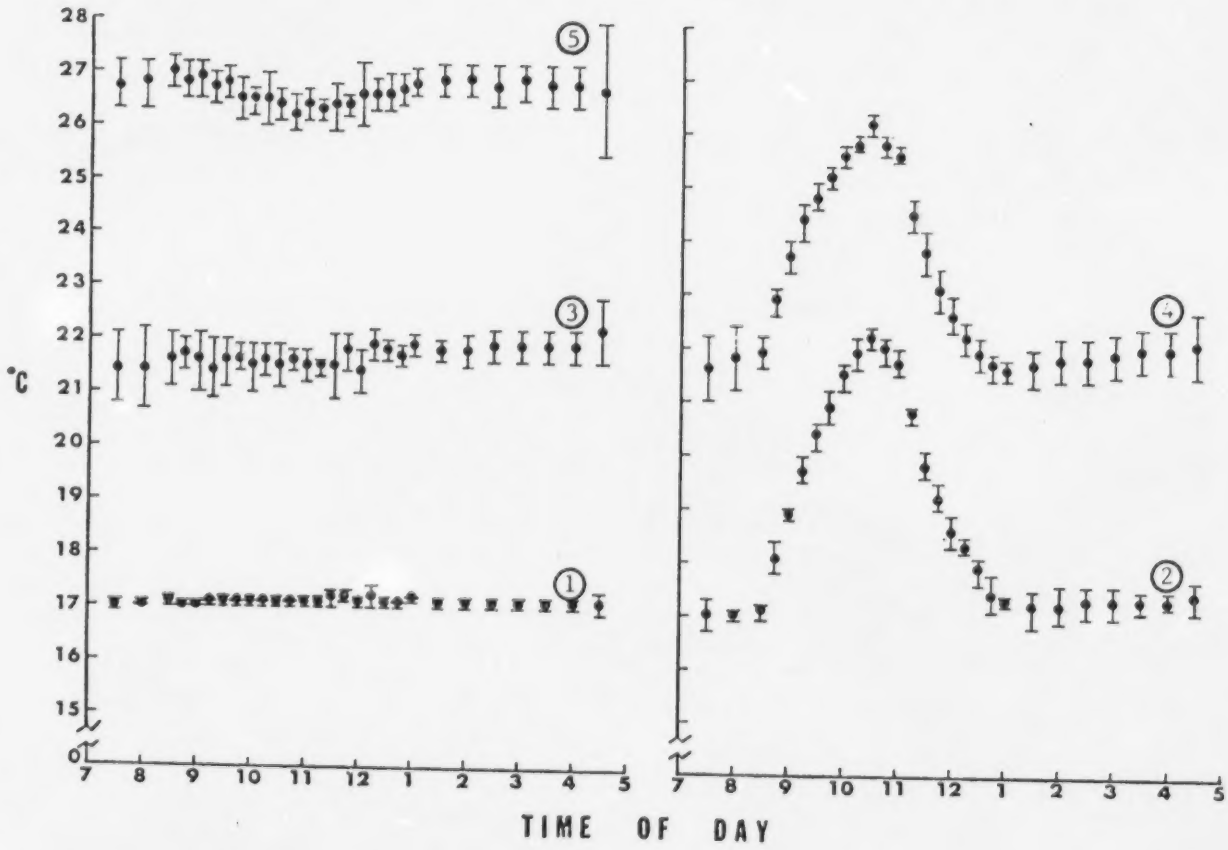

Figure 1. Experimental temperature profiles for constant and fluctuating temperature regimes.

TABLE 1. MANOVA TEST CRITERIA FOR THE HYPOTHESIS OF NO OVERALL EFFECT OF INCREASED TEMPERATURE.

	Groups tested								
	1,2,3	1,2	1,3	2,3	3,4,5	3,4	4,5	3,5	2,4
H = Type IV SS + CP Matrix									
E = Error SS + CP Matrix									
P = Dependent Variables	18	18	17	18	18	17	18	18	18
Q = Hypothesis DF	2	1	1	1	2	1	1	1	1
NE = DF of E	68	45	46	45	67	44	44	46	43
S = Min (P,Q)	2	1	1	1	2	1	1	1	1
M = 0.5 (ABS(P-Q)-1)	7.5	8.0	7.5	8.0	7.5	7.5	8.0	8.0	8.0
N = 0.5 (NE-P-1)	24.5	13.0	14.0	13.0	24.0	13.0	12.5	13.5	12.0
Wilk's Criterion	0.3834	0.6565	0.3086	0.3778	0.2629	0.3510	0.4717	0.2920	0.1964
Prob > F	0.0202	0.6706	0.0005	0.0124	0.0001	0.0044	0.1084	0.0006	0.0001

TABLE 2. RESULTS OF UNIVARIATE ANALYSIS OF VARIANCE TESTS.

	Groups tested								
	1,2,3	1,2	1,3	2,3	3,4,5	3,4	4,5	3,5	2,4
Hemoglobin									
Hematocrit									
ESR			*				*	*	
RBC								*	
MEV	*		**	*					
MEH	*		**	*					
MEHc									
Thrombocytes			*						*
Lymphocytes									*
Neutrophils	*	*	*						
Eosinophils			*	*					**
Basophils									
Hemoblasts									
Smudge cells	**		**	**	*	*	*		
Cell length			*	*	*	*		**	**
Cell width			*		**	*		**	*
Cell roundness					**	**		**	**
Cell area			*						

*P > 0.05; ** > 0.01.

TABLE 3. SUMMARY OF HEMATOLOGICAL RESPONSES BY GROUP.

	Group 1	Group 2	Group 3	Group 4	Group 5
Hemoglobin (HB) (g/100 ml)	5.52	5.79	5.98	5.94	6.18
Hematocrit (HC) (%)	33.56	35.91	37.63	38.06	41.02
Erythrocytic Sedimentation Rate (ESR) (mm/hr)	2.65	2.04	1.96	1.93	1.37
Number of Red Blood Cells (RBC) (cells/mm^3) x 10^6	2.25	2.21	2.05	2.09	2.34
Mean Erythrocyte Volume (MEV) (μ^3)	159.92	166.21	191.44	189.12	180.79
Mean Erythrocyte Hemoglobin (MEH) (pg)	25.12	26.69	30.42	29.23	27.05
Mean Erythrocyte Hemoglobin Concentration (MEHc) (%)	15.80	16.20	16.30	15.89	15.20
Thrombocytes (THR) (%)	60.29	62.17	65.21	69.14	68.04
Lymphocytes (LYM) (%)	31.50	33.17	30.25	27.11	28.60
Neutrophils (NEU) (%)	6.81	3.65	4.10	3.54	3.02
Eosinophils (EOS) (%)	1.12	0.96	0.35	0.11	0.19
Basophils (BAS) (%)	0.02	0.02	0.00	0.02	0.04
Hemoblasts (HEM) (%)	0.25	0.15	0.08	0.07	0.02
Smudge Cells (SMU) (%)	9.59	11.83	5.04	10.16	4.33
Cell Length (CL) (μ)	11.31	11.38	10.88	10.42	10.39
Cell Width (CW) (μ)	7.09	6.81	6.65	7.20	7.29
Cell Roundness (CR)	0.634	0.603	0.616	0.699	0.708
Cell Area (CA) (μ^2)	63.09	61.19	57.02	59.02	59.42
Fish Length (cm)	15.82	15.86	15.74	15.87	15.37

TABLE 4. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR GROUPS 1, 2, AND 3.

Variable	Percentage of the total variation	Cumulative percentage of the total variation
Smudge cells	18.23	18.23
MEH	10.09	28.32
MEV	9.69	38.01
Neutrophils	7.08	45.09
Cell length	6.59	51.68
Eosinophils	6.48	58.16
Cell area	6.20	64.36
ESR	5.42	69.78
Cell width	5.16	74.94
Thrombocytes	5.15	80.09
Cell roundness	3.75	83.84
RBC	3.59	87.43
Hemoglobin	3.54	90.97
Hemoblasts	2.89	93.86
Lymphocytes	2.52	96.38
Hematocrit	1.98	98.36
Basophils	1.19	99.55
MEHc	0.45	100.00

TABLE 5. RESULTS OF PARTIAL CORRELATIONS FOR GROUPS 1, 2, AND 3.

	HB	HC	ESR	RBC	MEV	MEH	MEHc	THR	LYM	NEU	EOS	BAS	HEM	SMU	CL	CW	CR	CA
HB						*	*											
HC							-*											
ESR																		
RBC					-*	-*												
MEV						*												
MEH							*											
MEHc																		
THR									-*	-*								
LYM																		
NEU													*					
EOS																		
BAS																		
HEM																		
SMU																		
CL																*		*
CW																	*	*
CR																		
CA																		

*P = 0.0001.

TABLE 6. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR GROUPS 1 AND 2.

Variable	Percentage of the total variation	Cumulative percentage of the total variation
Neutrophils	21.01	21.01
Cell roundness	16.61	37.62
ESR	14.27	51.89
Cell width	10.32	62.21
Smudge cells	8.23	70.44
Hemoglobin	4.83	75.27
MEH	4.64	79.91
Lymphocytes	4.10	84.01
Hemoblasts	3.39	87.40
Thrombocytes	3.13	90.53
MEV	3.00	93.53
Cell area	2.72	96.25
MEHc	1.32	97.57
Eosinophils	0.96	98.53
RBC	0.78	99.31
Cell length	0.43	99.74
Hematocrit	0.26	100.00
Basophils	0.00	100.00

TABLE 7. RESULTS OF PARTIAL CORRELATIONS FOR GROUPS 1 AND 2.

	HB	HC	ESR	RBC	MEV	MEH	MEHc	THR	LYM	NEU	EOS	BAS	HEM	SMU	CL	CW	CR	CA
HB						*												
HC																		
ESR																		
RBC					-*	-*												
MEV						*												
MEH							*											
MEHc																		
THR									-*	-*								
LYM																		
NEU													*					
EOS																		
BAS																		
HEM																		
SMU																		
CL																		*
CW																	*	*
CR																		
CA																		

*P = 0.0001.

TABLE 8. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR GROUPS 1 AND 3.

Variable	Percentage of the total variation	Cumulative percentage of the total variation
Smudge cells	13.64	13.64
MEH	10.82	24.46
MEV	9.46	33.92
Cell area	8.48	42.40
Eosinophils	8.43	50.83
Cell width	6.85	57.68
Thrombocytes	6.83	64.51
ESR	6.34	70.85
Neutrophils	6.08	76.93
Cell length	5.70	82.63
Hemoglobin	5.14	87.77
RBC	3.89	91.66
Hemoblasts	3.77	95.43
Hematocrit	2.02	97.45
Cell roundness	1.47	98.92
Lymphocytes	0.63	99.54
MEHc	0.46	100.00

TABLE 9. RESULTS OF PARTIAL CORRELATIONS FOR GROUPS 1 AND 3.

	HB	HC	ESR	RBC	MEV	MEH	MEHc	THR	LYM	NEU	EOS	BAS	HEM	SMU	CL	CW	CR	CA
HB																		
HC							-*											
ESR																		
RBC					-*	-*												
MEV						*												
MEH																		
MEHc																		
THR									-*									
LYM																		
NEU																		
EOS																		
HEM																		
SMU																		
CL																		*
CW																	*	*
CR																		
CA																		

*P = 0.0001.

TABLE 10. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR GROUPS 2 AND 3.

Variable	Percentage of the total variation	Cumulative percentage of the total variation
Smudge cells	33.12	33.12
Cell length	12.41	45.53
MEH	9.44	54.97
Eosinophils	9.05	64.02
MEV	8.88	72.90
Cell area	4.93	77.83
Lymphocytes	4.43	82.26
Thrombocytes	3.88	86.14
RBC	3.62	89.76
Basophils	2.43	92.19
Hematocrit	2.18	94.37
Cell roundness	1.54	95.91
Hemoblasts	1.29	97.20
Hemoglobin	1.17	98.37
Cell width	1.12	99.49
Neutrophils	0.34	99.83
ESR	0.13	99.96
MEHc	0.04	100.00

TABLE 11. RESULTS OF PARTIAL CORRELATIONS FOR GROUPS 2 AND 3.

	HB	HC	ESR	RBC	MEV	MEH	MEHc	THR	LYM	NEU	EOS	BAS	HEM	SMU	CL	CW	CR	CA
HB																		
HC							-*											
ESR																		
RBC					-*	-*												
MEV						*												
MEH																		
MEHc																		
THR									-*									
LYM																		
NEU													*					
EOS																		
BAS																		
HEM																		
SMU																		
CL																*		*
CW																	*	*
CR																		*
CA																		

*P = 0.0001.

TABLE 12. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR GROUPS 3, 4, AND 5.

Variable	Percentage of the total variation	Cumulative percentage of the total variation
Hematocrit	29.94	29.94
Cell roundness	20.62	50.56
Cell width	11.29	61.85
Smudge cells	6.56	68.41
Cell length	6.52	74.93
RBC	4.31	79.24
ESR	4.08	83.32
Eosinophils	2.92	86.24
MEH	2.89	89.13
Thrombocytes	1.78	90.91
Basophils	1.60	92.51
MEHc	1.35	93.86
Cell area	1.35	95.21
Hemoblasts	1.23	96.44
Neutrophils	1.21	97.65
Lymphocytes	1.14	98.79
Hemoglobin	0.64	99.43
MEV	0.57	100.00

TABLE 13. RESULTS OF PARTIAL CORRELATIONS FOR GROUPS 3, 4, AND 5.

	HB	HC	ESR	RBC	MEV	MEH	MEHc	THR	LYM	NEU	EOS	BAS	HEM	SMU	CL	CW	CR	CA
HB							*											
HC			-*		*		-*											
ESR																		
RBC					-*	-*												
MEV						*	*											
MEH																		
MEHc									-*									
THR																		
LYM																		
NEU																		
EOS														*				
BAS																		
HEM																		
SMU																		*
CL																	*	*
CW																		
CR																		
CA																		

*P = 0.0001.

TABLE 14. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR GROUPS 3 AND 4.

Variable	Percentage of the total variation	Cumulative percentage of the total variation
Cell roundness	40.30	40.30
Cell width	18.19	58.49
Cell length	11.28	69.77
Smudge cells	9.80	79.57
Eosinophils	7.40	86.97
Thrombocytes	4.51	91.48
Lymphocytes	3.40	94.88
Cell area	1.89	96.77
Neutrophils	1.13	97.90
MEH	0.96	98.86
MEHc	0.49	99.35
RBC	0.25	99.60
Hemoblasts	0.15	99.75
Hematocrit	0.14	99.89
MEV	0.06	99.95
Hemoblogin	0.04	99.99
ESR	0.01	100.00

TABLE 15. RESULTS OF PARTIAL CORRELATIONS FOR GROUPS 3 AND 4.

	HB	HC	ESR	RBC	MEV	MEH	MEHc	THR	LYM	NEU	EOS	HEM	SMU	CL	CW	CR	CA
HB																	
HC							-*										
ESR																	
RBC					-*	-*											
MEV						*											
MEH																	
MEHc																	
THR									-*								
LYM																	
NEU																	
EOS																	
HEM																	
SMU																	
CL																	*
CW																*	*
CR																	
CA																	

*P = 0.0001.

TABLE 16. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR GROUPS 4 AND 5.

Variable	Percentage of the total variation	Cumulative percentage of the total variation
Smudge cells	22.77	22.77
ESR	17.30	40.07
RBC	16.08	56.15
Hematocrit	11.07	67.22
MEH	7.21	74.43
Hemoblasts	5.71	80.14
MEHc	3.21	83.35
Eosinophils	2.86	86.21
Hemoglobin	2.63	88.84
Cell width	2.44	91.28
MEV	1.93	93.21
Neutrophils	1.64	94.85
Cell roundness	1.61	96.46
Lymphocytes	1.25	97.71
Basophils	1.19	98.90
Thrombocytes	0.62	99.52
Cell area	0.30	99.82
Cell length	0.18	100.00

TABLE 17. RESULTS OF PARTIAL CORRELATIONS FOR GROUPS 4 AND 5.

	HB	HC	ESR	RBC	MEV	MEH	MEHc	THR	LYM	NEU	EOS	BAS	HEM	SMU	CL	CW	CR	CA
HB																		
HC																		
ESR																		
RBC					-*	-*												
MEV						*	-*											
MEH																		
MEHc																		
THR									-*									
LYM																		
NEU																		
EOS																		
BAS																		
HEM																		
SMU																		
CL																	-*	*
CW																	*	*
CR																		
CA																		

*P = 0.0001.

TABLE 18. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR GROUPS 3 AND 5

Variable	Percentage of the total variation	Cumulative percentage of the total variation
Cell roundness	29.64	29.64
Cell width	15.05	44.69
Cell length	12.52	57.21
ESR	7.36	64.57
RBC	6.17	70.74
MEH	5.35	76.09
Hematocrit	4.37	80.46
Basophils	3.02	83.48
MEHc	2.55	86.03
Thrombocytes	2.50	88.53
Cell area	2.06	90.59
Eosinophils	2.02	92.61
Hemoblasts	2.01	94.62
Neutrophils	1.91	96.53
MEV	0.99	97.52
Smudge cells	0.95	98.47
Lymphocytes	0.78	99.25
Hemoglobin	0.75	100.00

TABLE 19. RESULTS OF PARTIAL CORRELATIONS FOR GROUPS 3 AND 5.

	HB	HC	ESR	RBC	MEV	MEH	MEHc	THR	LYM	NEU	EOS	BAS	HEM	SMU	CL	CW	CR	CA
HB																		
HC							-*											
ESR							*											
RBC					-*	-*												
MEV						*												
MEH																		
MEHc																		
THR									-*									
LYM																		
NEU																		*
EOS																		
BAS																		
HEM																		
SMU																		*
CL																	*	*
CW																		*
CR																		
CA																		

*P = 0.0001.

TABLE 20. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR GROUPS 2 AND 4.

Variable	Percentage of the total variation	Cumulative percentage of the total variation
Cell roundness	31.82	31.82
Cell length	19.05	50.87
Eosinophils	11.79	62.66
Lymphocytes	7.30	69.96
Thrombocytes	7.15	77.11
Cell width	6.23	83.34
MEV	5.37	88.71
MEH	3.15	91.86
Hematocrit	2.57	94.43
RBC	1.68	96.11
Hemoblasts	1.41	97.52
Cell area	1.12	98.64
Smudge cells	0.56	99.20
Hemoglobin	0.42	99.62
MEHc	0.24	99.86
ESR	0.13	99.99
Neutrophils	0.01	100.00
Basophils	0.00	100.00

TABLE 21. RESULTS OF PARTIAL CORRELATIONS FOR GROUPS 2 AND 4.

	HB	HC	ESR	RBC	MEV	MEH	MEHc	THR	LYM	NEU	EOS	BAS	HEM	SMU	CL	CW	CR	CA
HB		*																
HC																		
ESR																		
RBC					-*	-*												
MEV						*												
MEH																		
MEHc																		
THR									-*									
LYM																		
NEU																		
EOS																		
BAS														*				
HEM																		
SMU																		
CL																*		
CW																	*	*
CR																		
CA																		

*P = 0.0001.

TABLE 22. MANOVA TEST CRITERIA FOR THE HYPOTHESIS OF NO OVERALL EFFECT FOR INCREASED TEMPERATURE TREATMENT AND POWER PLANT EXPOSURE

	Increased Temperature	Intake vs Discharge
H = type IV SS & CP matrix		
E = error SS & CP matrix		
P = dependent variables	16	19
Q = hypothesis DF	1	1
NE = DF of E	38	26
S = min (P, Q)	1	1
M = 0.5 (ABS (P-Q)-1)	7.0	8.5
N = 0.5 (NE - P - 1)	10.5	3.0
Wilk's criterion	0.2738	0.1211
Prob >F	0.0018	0.0549

TABLE 23. RESULTS OF UNIVARIATE ANALYSIS OF VARIANCE TESTS

	Increased Temperature	Intake vs Discharge
Hemoglobin		**
Hematocrit		*
ESR		
RBC		*
MEV		
MEH		
MEHc	*	**
Cell height		
Cell length	**	**
Cell width		*
Cell roundness	**	**
Cell area		
Thrombocytes	*	
Lymphocytes	**	
Neutrophils		
Eosinophils		
Hemoblasts		
Basophils		
Total protein		
Hepatosomatic Index		
Fish length		

*P >0.05; **P >0.01

TABLE 24. SUMMARY OF HEMATOLOGICAL RESPONSES FOR INCREASED TEMPERATURE AND POWER PLANT EXPOSURE

	12.4°C	20.°C	Intake	Discharge
Hemoglobin (HB) (g/100ml)	5.84	5.46	7.24	4.95
Hematocrit (HC) (%)	43.74	45.76	44.97	39.40
Erythrocytic Sedimentation Rate (ESR) (mm/hr)	1.64	1.23	2.78	3.23
Number of Red Blood Cells (RBC) (cell/mm^3) x 10^6	2.71	2.69	2.45	1.92
Mean Erythrocyte Volume (MEV) (μ^3)	165.11	184.81	215.14	210.07
Mean Erythrocyte Hemoglobin (MEH) (pg)	22.20	21.90	34.02	26.23
Mean Erythrocyte Hemoglobin concentration (MEHc) (%)	13.48	11.98	16.19	12.58
Cell Height (CH) (μ)	3.58	4.17	4.50	4.20
Cell Length (CL) (μ)	12.00	11.14	12.18	11.33
Cell Width (CW) (μ)	7.38	7.70	7.81	8.49
Cell Roundness (CR)	0.621	0.698	0.644	0.756
Cell Area (CA) (μ^2)	69.53	67.29	74.80	75.63
Thrombocytes (THR) (%)	89.60	87.10	80.39	84.18
Lymphocytes (LYM) (%)	8.70	11.95	14.96	13.14
Neutrophils (NEU) (%)	1.65	0.85	3.43	2.32
Eosinophils (EOS) (%)	0.02	0.02	0.18	0.18
Hemoblasts (HEM) (%)	0.02	0.08	0.32	0.07
Basophils (BAS) (%)	-	-	0	0.04
Hepatosomatic Index (HSI) (%)	-	-	0.73	0.74
Fish Length (L) (cm)	13.83	13.84	11.68	11.68

TABLE 25. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR INCREASED TEMPERATURE TREATMENT

Variable	Percentage of the Total Variation	Cumulative percentage of the Total Variation
Cell length	23.00	23.00
Cell roundness	20.71	43.71
Lymphocytes	13.48	57.19
MEHc	7.64	64.83
Thrombocytes	6.15	70.98
Cell width	5.14	76.12
Cell height	5.10	81.22
Neutrophils	3.65	84.87
MEV	3.63	88.50
Hemoglobin	3.16	91.66
ESR	2.74	94.40
Hematocrit	2.46	96.86
Cell area	2.08	98.94
Hemoblasts	1.00	99.94
MEH	0.05	99.99
RBC	0.01	100.00

TABLE 26. RESULTS OF PARTIAL CORRELATIONS FOR INCREASED TEMPERATURE TREATMENT.

	HB	HC	ESR	RBC	MEV	MEH	MEHc	CH	CL	CW	CR	CA	THR	LYM	NEU	HEM
HB							*									
HC																
ESR																
RBC					-*	-*		-*								
MEV						*		*								
MEH								*								
MEHc																
CH																
CL											-*	*				
CW											*	*				
CR																
CA																
THR														-*	-*	
LYM																
NEU																
HEM																

* P = 0.0001

TABLE 27. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR POWER PLANT EXPOSURE

Variable	Percentage of the Total Variation	Cumulative percentage of the Total Variation
Cell roundness	19.42	19.42
Hemoglobin	17.81	37.23
MEHc	12.73	49.96
Cell length	12.43	62.39
Cell width	9.31	71.70
Hematocrit	8.49	80.19
RBC	5.70	85.89
Hemoblasts	3.82	89.71
Thrombocytes	3.09	92.80
MEH	2.03	94.83
Neutrophils	1.69	96.52
Lymphocytes	1.54	98.06
Basophils	1.03	99.09
ESR	0.64	99.73
Cell area	0.12	99.85
Cell height	0.10	99.95
HSI	0.03	99.98
MEV	0.02	100.00

TABLE 28. RESULTS OF PARTIAL CORRELATIONS FOR POWER PLANT EXPOSURE

	HB	HC	ESR	RBC	MEV	MEH	MEHc	CH	CL	CW	CR	CA	THR	LYM	MEU	HEM	HSI
HB							*										
HC																	
ESR																	
RBC					-*	-*		-*									
MEV						*		*									
MEH								*									
MEHc																	
CH																	
CL												*					
CW											*	*					
CR																	
CA																	
THR														-*			
LYM																	
NEU																	
HEM																	
HSI																	

* P = 0.0001